

What is claimed is:

1. (Currently Amended) A method of identifying ~~one or more markers~~ a marker useful for detecting Alzheimer's Disease, said method wherein each of said one or more markers corresponds to a gene transcript, comprising the steps of:
 - a) using an oligonucleotide of predetermined sequence, detecting a presence in RNA of blood samples which have not been fractionated into cell types from subjects having Alzheimer's Disease, of RNA encoded by a gene, said gene expressed in blood and in a non-blood tissue of a subject not having Alzheimer's Disease, said oligonucleotide being specific only for RNA, or cDNA complementary to said RNA, encoded by said gene in said samples, determining the level of one or more gene transcripts expressed in blood obtained from one or more individuals having Alzheimer's Disease, wherein each of said one or more transcripts is expressed by a gene that is a candidate marker for Alzheimer's Disease; and
 - b) quantifying a level of said RNA encoded by said gene; and comparing the level of each of said one or more gene transcripts from said step a) with the level of each of said one or more genes transcripts in blood obtained from one or more individuals not having Alzheimer's Disease;
 - c) determining a statistically significant difference between said quantified level and a quantified level of a control RNA encoded by said gene in RNA of blood samples which have not been fractionated into cell types from control subjects, said control RNA having been detected in said samples from said control subjects, thereby identifying said gene as being a marker useful for detecting Alzheimer's Disease. wherein those compared transcripts which display differing levels in the comparison of step b) are identified as being markers for Alzheimer's Disease.
2. (Currently Amended) A method of identifying ~~two~~ one or more markers useful for detecting Alzheimer's Disease, said method wherein each of said one or more markers corresponds to a gene transcript, comprising the steps of:
for each of a collection of two or more genes;

a) using an oligonucleotide of predetermined sequence, detecting a presence in RNA of blood samples which have not been fractionated into cell types from subjects having Alzheimer's Disease, of RNA encoded by said gene, said oligonucleotide being specific only for RNA, or cDNA complementary to said RNA, encoded by said gene in said samples, said gene being expressed in blood and in a non-blood tissue of a subject not having Alzheimer's Disease;

~~determining the level of one or more gene transcripts expressed in blood obtained from one or more individuals having Alzheimer's Disease, wherein each of said one or more transcripts is expressed by a gene that is a candidate marker for Alzheimer's Disease; and~~

b) quantifying a level of said RNA encoded by said gene; and comparing the level of each of said one or more gene transcripts from said step a) with the level of each of said one or more genes transcripts in blood obtained from one or more individuals having Alzheimer's Disease;

c) determining a statistically significant difference between said quantified level and a quantified level of control RNA encoded by said gene in RNA of blood samples which have not been fractionated into cell types from control subjects, said control RNA having been detected in said samples from said control subjects,

thereby identifying said two or more genes as two or more markers useful in detecting Alzheimer's Disease.

~~wherein those compared transcripts which display the same levels in the comparison of step b) are identified as being markers for Alzheimer's Disease.~~

3.(currently amended) A method of identifying ~~one or more markers of a stage of a~~ marker useful for detecting Alzheimer's Disease, said method comprising: ~~Alzheimer's Disease progressio or regression, wherein each of said one or more markers corresponds to a gene transcript, comprising the steps of:~~

a) producing amplification products from RNA of blood samples which have not been fractionated into cell types, from subjects having Alzheimer's Disease, using primers specific only for RNA, and/or cDNA complementary to said RNA, encoded by a gene in said samples, said gene being expressed in blood and in a non-blood tissue of a subject not having Alzheimer's Disease; determining the

~~level of one or more gene transcripts expressed in blood obtained from one or more individuals having a stage of Alzheimer's Disease, wherein said one or more individuals are at the same progressive or regressive stage of Alzheimer's Disease, and wherein each of said one or more transcripts is expressed by a gene that is a candidate marker for determining the stage of progression or regression of Alzheimer's Disease, and;~~

~~b) quantifying a level of said amplification products; and comparing the level of each of said one or more gene transcripts from said step a) with the level of each of said one or more genes transcripts in blood obtained from one or more individuals who are at a progressive or regressive stage of Alzheimer's Disease distinct from that of said one or more individuals of step a);~~

~~c) determining a statistically significant difference between said quantified level of said amplification products and a quantified level of amplification products produced using primers specific only for RNA, and/or cDNA complementary to said RNA, encoded by said gene from control RNA, in RNA of blood samples which have not been fractionated into cell types, said control RNA having been detected in said samples from said control subjects,~~

~~thereby identifying said gene as being a marker useful for detecting Alzheimer's Disease,~~

~~wherein those compared transcripts which display differing levels in the comparison of step~~

~~b) are identified as being markers for the stage of progression or regression of Alzheimer's Disease.~~

4.(currently amended) A method of identifying ~~two~~ one or more markers ~~of a stage of Alzheimer's Disease progression or regression, wherein each of said one or more markers corresponds to a gene transcript, useful for detecting Alzheimer's Disease, said method comprising the steps of:~~

for each of a collection of two or more genes;

a) producing amplification products from RNA of blood samples which have not been fractionated into cell types from subjects having Alzheimer's Disease, using primers specific only for RNA, and/or cDNA complementary to said RNA, encoded by said gene of said subjects, wherein said gene is expressed in blood and in a non-blood tissue of a subject not having Alzheimer's Disease; ~~said~~

~~determining the level of one or more gene transcripts expressed in blood obtained from one or more individuals having a stage of Alzheimer's Disease, wherein said one or more individuals are at the same progressive or regressive stage of Alzheimer's Disease, and wherein each of said one or more transcripts is expressed by a gene that is a candidate marker for determining the stage of progression or regression of Alzheimer's Disease, and;~~

~~b) quantifying a level of said amplification products; and comparing the level of each of said one or more gene transcripts from said step a) with the level of each of said one or more genes transcripts in blood obtained from one or more individuals who are at a progressive or regressive stage of Alzheimer's Disease identical to that of said one or more individuals of step a);~~

~~c) Determining a statistically significant difference between said quantified level of said amplification products and a quantified level of amplification products produced using primers specific only for RNA, and/or cDNA complementary to said RNA, encoded by said gene, from control RNA in RNA of blood samples which have not been fractionated into cell types, from control subjects, said control RNA having been detected in said samples from said control subjects, thereby identifying said collection of said two or more genes as two or more markers useful for detecting Alzheimer's Disease.~~

~~wherein those compared transcripts which display the same levels in the comparison of step b) are identified as being markers for the stage of progression or regression of Alzheimer's Disease.~~

5. (currently amended) The method of any one of claims 1-4, wherein each of said one or more markers ~~identifies one or more transcripts of one or more~~ corresponds to a non immune response genes.

6. (canceled)

7. (currently amended) The method of any one of claims 1-4, wherein each of said one or more markers corresponds to a gene expressed ~~identifies a transcript of a gene expressed by~~ non-lymphoid tissue.

8. (currently amended) The method of any one of claims 1-4, wherein ~~said~~ one marker of

said one or more markers identifies the sequence amyloid precursor protein (APP).

9. (currently amended) A method of detecting a difference in expression of a gene in a human test subject as compared with human control subjects, said method comprising: ~~diagnosing or prognosing Alzheimer's Disease in an individual, comprising the steps of:~~
- a) using an oligonucleotide of predetermined sequence, detecting in RNA of a blood sample from said test subject which has not been fractionated into cell types, RNA encoded by said gene in said sample, wherein said gene is expressed in blood and in a non-blood tissue in a subject who is not said test subject, said oligonucleotide being specific only for RNA or cDNA complementary to said RNA, encoded by said gene; ~~determining the level of one or more gene transcripts expressed in blood obtained from said individual, wherein said one or more gene transcripts corresponds to said one or more markers of claim 1 and claim 2, and~~
 - b) quantifying a level of said RNA encoded by said gene; and ~~comparing the level of each of said one or more gene transcripts in said blood according to step a) with the level of each of said one or more gene transcripts in blood from one or more individuals not having Alzheimer's Disease,~~
 - c) determining a statistically significant difference between said level and a quantified level of control RNA encoded by said gene in RNA of blood samples which have not been fractionated into cell types from said control subjects, wherein said difference is indicative of Alzheimer's Disease in said test subject, thereby detecting a difference in expression of said gene in said human test subject vs. said human control subjects.

~~wherein detecting a difference in the levels of each of said one or more gene transcripts in the comparison of step b) is indicative of Alzheimer's Disease in the individual of step a).~~

10. (currently amended) A method of detecting a difference in expression of each of two or more genes of human test subjects vs. human control subjects comprising: ~~diagnosing or prognosing Alzheimer's Disease in an individual, comprising the steps of:~~

For each gene of a collection of said two or more genes:

- a) using an oligonucleotide of predetermined sequence, detecting in RNA of a blood sample from said test subject which has not been fractionated into cell

types, RNA encoded by said gene in said sample, wherein said gene is expressed in blood and in a non-blood tissue in a subject who is not said test subject, said oligonucleotide being specific only for RNA, or cDNA complementary to said RNA, encoded by said gene; determining the level of one or more gene transcripts expressed in blood obtained from said individual, wherein said one or more gene transcripts correspond to said one or more markers of claim 1 and claim 2 and
b) quantifying a level of said amplification product; and comparing the level of each of said one or more gene transcripts in said blood according to step a) with the level of each of said one or more gene transcripts in blood from one or more individuals having Alzheimer's Disease,
c) determining a statistically significant difference between said level and a quantified level of control RNA encoded by said gene in RNA of blood samples which have not been fractionated into cell types from said one or more control subjects, said control RNA having been detected in said samples for said control subjects; wherein said difference for each said gene is indicative of Alzheimer's Disease in said test subject,

thereby detecting a difference in expression of each said gene in said collection of two or more genes in blood of said human test subjects vs. said human control subjects.

~~wherein detecting the same levels of each of said one or more gene transcripts in the comparison of step b) is indicative of Alzheimer's Disease in the individual of step a).~~

11. (currently amended) A method of detecting a difference in expression of a gene of a human test subject vs. human control subjects, said method determining a stage of disease progression or regression in an individual having Alzheimer's Disease, comprising the steps of:

a) producing amplification products from RNA of a blood sample from said test subject which has not been fractionated into cell types, using primers specific only for RNA and/or cDNA complementary to said RNA, encoded by said gene, wherein said gene is expressed in blood and in a non-blood tissue of a subject who is not said test subject; determining the level of one or more gene transcripts expressed in blood obtained from said individual having Alzheimer's Disease, wherein said one or more gene transcripts correspond to said one or more markers

~~of claim 3 and claim 4, and~~

~~b) quantifying a level of said amplification product; and comparing the level of each if said one or more gene transcripts in said blood according to step a) with the level of each of said one or more gene transcripts in said blood obtained from one or more individuals who each have been diagnosed as being at the same progressive or regressive stage of Alzheimer's Disease;~~

c) determining a statistically significant difference between said quantified level of said amplification products and a quantified level of amplification products produced using primers specific only for RNA and/or cDNA complementary to said RNA, encoded by said gene, applied to control RNA of blood samples which have not been fractionated into cell types from said control subjects, wherein detection of said difference for said gene is indicative of Alzheimer's Disease in said test subject,

thereby detecting a difference in expression of said gene of said human test subject vs. said human control subjects.

~~wherein the comparison from step b) allows the determination of the stage of Alzheimer's Disease progression or regression in the individual of step a).~~

12. (Currently amended) A method of ~~diagnosing or prognosing Alzheimer's Disease in an individual, comprising the steps of:~~ detecting a difference in expression of each of two or more genes of a human test subject vs. human control subjects, said method comprising:
for each gene of said collection of two or more genes:

a) producing an amplification product from RNA of a blood sample from said test subject which has not been fractionated into cell types, using primers specific only for RNA and/or cDNA complementary to said RNA, encoded by said gene, wherein said gene is expressed in blood and in a non-blood tissue of a subject who is not said test subject; and determining the level of one or more gene transcripts expressed in blood obtained from said individual, wherein said one or more gene transcripts corresponds to said one or more markers of claim 1 and claim 2, and

b) quantifying a level of said amplification product, comparing the level of each of said one or more gene transcripts in said blood according to step a) with the

~~level of each of said one or more gene transcripts in blood from one or more individuals having Alzheimer's Disease;~~

c) determining a statistically significant difference between said quantified level of said amplification product and a quantified level of amplification products produced using primers specific only for RNA and/or cDNA complementary to said RNA, encoded by said gene applied to control RNA of blood samples which have not been fractionated into cell types from said control subjects, said control RNA having been detected in said samples from said control subjects, wherein determining said statistically significant difference for each said gene is indicative of Alzheimer's Disease in said test subject, comparing the level of each of said one or more gene transcripts in said blood according to step a) with the level of each of said one or more gene transcripts in blood from one or more individuals not having Alzheimer's Disease

d) ~~determining whether the level of said one or more gene transcripts of step a) classify with the levels of said transcripts in step b) as compared with levels of said transcripts in step c);~~

thereby detecting a difference in expression of each said gene in said collection of two or more genes in blood of said human test subject vs. said human control subjects.

~~wherein said determination is indicative of said individual of step a) having Alzheimer's Disease.~~

13. (canceled)

14.-15 (Canceled)

16.-18. (canceled)

19. (currently amended)The method of any one of claims 1-4 and 9-12, 15, further comprising the step of isolating RNA from said ~~blood~~ samples.

20. (currently amended)The method of any one of claims 1-4 and 9-12, 1-4 and 9-13, wherein the step said steps of determining said levels of RNA encoded by said gene in step (a) and/or step (b) is effected using the level of each of said one or more gene transcripts comprises quantitative RT-PCR (QRT-PCR); ~~wherein said one or more transcripts are from step a) and/or step b) of claims 1-4 and 9-13.~~

21.(Canceled)

22.(Currently amended) The method of any one of claims 1-4 and 9-12, ~~claim 20~~, wherein said primers are 15-25 nucleotides in length.

23.(Currently amended) The method of any one of claims 1-4 and ~~9-12~~ 9-13, wherein the step of determining said levels of RNA encoded by each of said genes in step (a) and/or step (b) is by the level of each of said one or more gene transcripts ~~comprises~~ hybridizing a first plurality of isolated nucleic acid molecules that correspond to said genes ~~one or more transcripts~~, to an array comprising a second plurality of isolated nucleic acid molecules.

24.(Original) The method of claim 23, wherein said first plurality of isolated nucleic acid molecules comprises RNA, DNA, cDNA, PCR products or ESTs.

25.(Original) The method of claim 23, wherein said array comprises a plurality of isolated nucleic acid molecules comprising RNA, DNA, cDNA, PCR products or ESTs.

26.(Canceled)

27.(Currently amended) The method of claim ~~25~~-23, wherein said array comprises two or more of the markers of claim 2.

28.(Currently amended) The method of claim ~~25~~-23, wherein said array comprises two or more of the markers of claim 3.

29.(Currently amended) The method of claim ~~25~~-23, wherein said array comprises two or more of the markers of claim 4.

30.(Currently amended) The method of claim ~~25~~-23, wherein said array comprises a plurality of nucleic acid molecules that correspond to genes of the human genome.

31. (original) A plurality of isolated nucleic acid molecules that correspond to two or more of the markers of claim 1.

32. (original) A plurality of isolated nucleic acid molecules that correspond to two or more of the markers of claim 2.

33. (original) A plurality of isolated nucleic acid molecules that correspond to two or more of the markers of claim 3.

34. (original) A plurality of isolated nucleic acid molecules that correspond to two or more of the markers of claim 4.

35. (canceled)

36. (original) An array consisting essentially of the plurality of nucleic acid molecules of claim 31.

37. (original) An array consisting essentially of the plurality of nucleic acid molecules of claim 32.

38. (original) An array consisting essentially of the plurality of nucleic acid molecules of claim 33.

39. (original) An array consisting essentially of the plurality of nucleic acid molecules of claim 34.

40. (original) A kit for diagnosing or prognosing Alzheimer's Disease comprising:

- a) two gene-specific priming means designed to produce double stranded DNA complementary to a gene that corresponds to a marker selected from the group consisting of markers of claim 1, claim 2, claim 3, and claim 4; wherein said first priming means contains a sequence which can hybridize to RNA, cDNA or an EST complementary to said gene to create an extension product and said second priming means capable of hybridizing to said extension product;
- b) an enzyme with reverse transcriptase activity,
- c) an enzyme with thermostable DNA polymerase activity, and
- d) a labeling means;

wherein said primers are used to detect the quantitative expression levels of said gene in a test subject.

41. (original) A kit for monitoring a course of therapeutic treatment of Alzheimer's Disease, comprising:

- a) two gene-specific priming means designed to produce double stranded DNA complementary to a gene that corresponds to a marker selected from the group consisting of markers of claim 1, claim 2, claim 3, and claim 4; wherein said first priming means contains a sequence which can hybridize to RNA, cDNA or an EST complementary to

said gene to create an extension product and said second priming means capable of hybridizing to said extension product;

b) an enzyme with reverse transcriptase activity,

c) an enzyme with thermostable DNA polymerase activity, and

d) a labeling means;

wherein said primers are used to detect the quantitative expression levels of said gene in a test subject.

42. (original) A kit for monitoring progression or regression of Alzheimer's Disease, comprising:

a) two gene-specific priming means designed to produce double stranded DNA complementary to a gene that corresponds to a marker selected from the group consisting of markers of claim 1, claim 2, claim 3, and claim 4, wherein said first priming means contains a sequence which can hybridize to RNA, cDNA or an EST complementary to said gene to create an extension product and said second priming means capable of hybridizing to said extension product;

b) an enzyme with reverse transcriptase activity,

c) an enzyme with thermostable DNA polymerase activity, and

d) a labeling means;

wherein said primers are used to detect the quantitative expression levels of said gene in a test subject.

43. (original) The method of claim 25, wherein said ESTs comprise a length greater than 100 nucleotides.

44. (new) A method of identifying a marker useful for detecting Alzheimer's Disease, said method comprising:

a) using an oligonucleotide of predetermined sequence, detecting a presence in RNA of unfractionated cells of a lysed blood sample from subjects having Alzheimer's Disease, of RNA encoded by a gene, said gene expressed in blood and in a non-blood tissue of a subject not having Alzheimer's Disease, said oligonucleotide being specific only for RNA, or cDNA complementary to said RNA, encoded by said gene in said samples,

b) quantifying a level of said RNA encoded by said gene; and

c) determining a statistically significant difference between said quantified level and a quantified level of a control RNA encoded by said gene in RNA of unfractionated cells of a lysed blood sample from control subjects, said control RNA having been detected in said samples from said control subjects,

thereby identifying said gene as being a marker useful for detecting Alzheimer's Disease.

45. (new) A method of identifying two or more markers useful for detecting Alzheimer's Disease, said method comprising:

for each of a collection of two or more genes

a) using an oligonucleotide of predetermined sequence, detecting a presence in RNA of unfractionated cells of a lysed blood sample from subjects having Alzheimer's Disease of RNA encoded by said gene, said oligonucleotide being specific only for RNA, or cDNA complementary to said RNA, encoded by said gene in said samples, said gene being expressed in blood and in a non-blood tissue of a subject not having Alzheimer's Disease;

b) quantifying a level of said RNA encoded by said gene; and

c) determining a statistically significant difference between said quantified level and a quantified level of control RNA encoded by said gene in RNA of unfractionated cells of a lysed blood sample from control subjects, said control RNA having been detected in said samples from said control subjects,

thereby identifying said two or more genes as two or more markers useful in detecting Alzheimer's Disease .

46. (new) A method of identifying a marker useful for detecting Alzheimer's Disease, said method comprising:

a) producing amplification products from RNA of unfractionated cells of a lysed blood sample, from subjects having Alzheimer's Disease, using primers specific only for RNA, and/or cDNA complementary to said RNA, encoded by a gene in said samples, said gene being expressed in blood and in a non-blood tissue of a subject not having Alzheimer's Disease;

b) quantifying a level of said amplification products; and

c) determining a statistically significant difference between said quantified level of said amplification products and a quantified level of amplification products produced using primers specific only for RNA, and/or cDNA complementary to said RNA, encoded by said gene from control RNA, in RNA of unfractionated cells of a lysed blood sample, said control RNA having been detected in said samples from said control subjects, thereby identifying said gene as being a marker useful for detecting Alzheimer's Disease.

47. (new)A method of identifying two or more markers useful for detecting Alzheimer's Disease, said method comprising:
for each of a collection of two or more genes:

a) producing amplification products from RNA of unfractionated cells of a lysed blood sample from subjects having Alzheimer's Disease, using primers specific only for RNA, and/or cDNA complementary to said RNA, encoded by said gene of said subjects, wherein said gene is expressed in blood and in a non-blood tissue of a subject not having Alzheimer's Disease;

b) quantifying a level of said amplification products; and

c) Determining a statistically significant difference between said quantified level of said amplification products and a quantified level of amplification products produced using primers specific only for RNA, and/or cDNA complementary to said RNA, encoded by said gene, from control RNA in RNA of unfractionated cells of a lysed blood sample, from control subjects, said control RNA having been detected in said samples from said control subjects,

thereby identifying said collection of said two or more genes as two or more markers useful for detecting Alzheimer's Disease.

48. (new)A method of detecting a difference in expression of a gene in a human test subject as compared with human control subjects, said method comprising:

a) using an oligonucleotide of predetermined sequence, detecting in RNA of a unfractionated cells of a lysed blood sample of said test subject, RNA encoded by said gene in said sample, wherein said gene is expressed in blood and in a non-blood tissue in

a subject who is not said test subject, said oligonucleotide being specific only for RNA or cDNA complementary to said RNA, encoded by said gene;

b) quantifying a level of said RNA encoded by said gene; and

c) determining a statistically significant difference between said level and a quantified level of control RNA encoded by said gene in RNA of unfractionated cells of a lysed blood sample from said control subjects, wherein said statistically significant difference is indicative of Alzheimer's Disease in said test subject,

thereby detecting a difference in expression of said gene in said human test subject vs. said human control subjects.

49. (new) A method of detecting a difference in expression of each of two or more genes of a human test subjects vs. human control subjects:

for each gene of a collection of said two or more genes:

a) using an oligonucleotide of predetermined sequence, detecting in RNA of a unfractionated cells of a lysed blood sample from said test subject, RNA encoded by said gene in said sample, wherein said gene is expressed in blood and in a non-blood tissue in a subject who is not said test subject, said oligonucleotide being specific only for RNA, or cDNA complementary to said RNA, encoded by said gene;

b) quantifying a level of said RNA encoded by said gene; and

c) determining a statistically significant difference between said level and a quantified level of control RNA encoded by said gene in RNA of unfractionated cells of a lysed blood sample from said control subjects, said control RNA having been detected in said samples for said control subjects; wherein said statistically significant difference for each said gene is indicative of Alzheimer's Disease in said test subject,

thereby detecting a difference in expression of each said gene in said collection of two or more genes in blood of said human test subjects vs. said human control subjects.

50. (new) A method of detecting a difference in expression of a gene of a human test subject vs. human control subjects, said method comprising:

- a) producing amplification products from RNA of a blood sample unfractionated cells of a lysed blood sample from said test subject, using primers specific only for RNA and/or cDNA complementary to said RNA, encoded by said gene, wherein said gene is expressed in blood and in a non-blood tissue of a subject who is not said test subject;
- b) quantifying a level of said amplification product; and
- c) determining a statistically significant difference between said quantified level of said amplification products and a quantified level of amplification products produced using primers specific only for RNA and/or cDNA complementary to said RNA, encoded by said gene, applied to control RNA of unfractionated cells of a lysed blood sample from said control subjects, wherein detection of said statistically significant difference for said gene is indicative of Alzheimer's Disease in said test subject,

thereby detecting a difference in expression of said gene in blood of said human test subject vs. human control subjects.

51. (New) A method of detecting a difference in expression of each of two or more genes of a human test subject vs. human control subjects, said method comprising:
for each gene of said collection of two or more genes:

- a) producing an amplification product from RNA of a unfractionated cells of a lysed blood sample from said test subject, using primers specific only for RNA and/or cDNA complementary to said RNA, encoded by said gene, wherein said gene is expressed in blood and in a non-blood tissue of a subject who is not said test subject; and
- b) quantifying a level of said amplification product,
- c) determining a statistically significant difference between said quantified level of said amplification product and a quantified level of amplification products produced using primers specific only for RNA and/or cDNA complementary to said RNA, encoded by said gene applied to control RNA of unfractionated cells of a lysed blood sample from said control subjects, said control RNA having been detected in said samples from said control subjects, wherein detecting said statistically significant difference for each said gene is indicative of Alzheimer's Disease in said test subject,

thereby detecting a difference in expression of each said gene in said collection of two or more genes in blood of said human test subjects vs. said human control subjects.